Synopsis of Ph.D. Research Work Entitled

Ameliorating effects of *Aloe vera* on Fluoride induced toxicity on *Channa punctatus* (Bloch) from Biochemical responses to Underlying Integrative Networks.

To be undertaken by

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State of Art

Fluoride is a naturally occurring element found in various environmental compartments, including water, soil, and air. While fluoride is beneficial in small amounts, especially for dental health in humans, elevated concentrations can be toxic to aquatic life, including fish (Camargo et al.,2003). The study of fluoride toxicity in fish is essential for understanding the ecological impacts of industrial discharges, agricultural runoff, and natural geochemical processes that elevate fluoride levels in aquatic systems (Wang et al., 2019)

Fluoride enters aquatic systems from both natural and anthropogenic sources. The toxic effects of fluoride on fish encompass a range of physiological, biochemical, and behavioral changes like Alterations in gill structure, impaired respiration, and disruptions in osmoregulation. Behavioral effects includes reduced swimming performance, and increases susceptibility to predators (Dey, D et al.,2015).

Water hardness, pH, and temperature can modulate fluoride toxicity. Harder water with higher calcium levels can mitigate fluorides effects.

Regulatory framework may include safe fluoride concentration limits in aquatic environments to protect fish and other aquatic organisms. Implementing practices to reduce fluoride discharge from industrial and agricultural sources.

Aloe vera, a succulent plant species of the genus Aloe, is widely recognized for its medicinal and therapeutic properties. Recent studies have explored its potential benefits in aquaculture, particularly in enhancing the health and well -being of fish.

Ongoing research efforts are focused on elucidating the complex interactions between fluoride and fish physiology with the aim of developing effective regulatory and mitigation strategies to manage fluoride pollution.

A study on Nile tilapia revealed that dietary Aloe Vera significantly increased lysozyme activity and serum bactericidal activity, indicating enhanced immune function (Ahmed et al.,2019). It was also found that Aloe vera supplementation led to improved growth parameters and feed efficiency.

The use of Aloe Vera in aquaculture presents a promising natural alternative to enhance fish health, improve water quality and promote sustainable fish Farming practices. Future research should focus on optimizing dosages, understanding long term effects, and exploring the synergistic effects of Aloe vera into aquaculture can potentially lead to healthier fish population, reduced reliance on antibiotics, and more sustainable aquaculture practices.

PROBLEM TO BE INVESTIGATED:

Research envisages development of a stimulated system in which the test species will be treated with sublethal dose of Sodium fluoride in acidified ambient water by an acute toxicity test. Fish will be divided into control and treatment groups. The treatment groups will be exposed to sub lethal concentrations of fluoride over a specified period.

In the present study the following activities will be taken up in freshwater fish *Channa* with metal toxicity:

1. Enzyme activities will be measured.
2. Level of Oxidative stress markers will be assessed.
3. Histological studies; Tissues samples from liver, gills, and kidney will be examined for histopathological changes.
4. Measurement of Haematological parameters.
5. SEM of gill tissues , liver and kidney will be analyzed .

OBJECTIVE OF THE INVESTIGATION:

The primary objective of this study is to investigate the effects of fluoride induced toxicity on Channa punctatus and to evaluate the consequent changes in specific biochemical parameters. To assess the survival and Behavioral changes on fish, to Evaluate the biochemical parameters, Oxidative stress markers. Biochemical, Hematological and SEM and Histopathological studies will also be done to measure the stress level and recovery of fish.

HYPOTHESIS:

Sodium fluoride has been recognized as the powerful toxicant posing a threat to aquatic organism including fish at low pH I soft water.

Exposure to sub lethal concentrations of fluoride induced significant biochemical alterations in Channa punctatus, which can be mitigated by the therapeutic use of Aloe vera extract, thereby enhancing the fish recovery potential.

Status of Research:

Research has documented species specific responses to fluoride exposure, with some species being more sensitive than others. Chronic exposure often leads to more severe effects compared to acute exposure. There is a growing interest in understanding the molecular and genetic responses of fish to fluoride stress, with some studies focusing on the expression of stress related genes and proteins. The Potential of Aloe Vera to mitigate oxidative stress and enhance immune responses make it a candidate for aiding recovery in fluoride exposed fish.

International Status:

Camargo, J.A. (2003). Fluoride toxicity to aquatic organisms: A Review. Chemosphere, 50(3),251-264. This article provides a detailed examination of the toxic effects of fluoride on various aquatic organisms, including fish.

National Status:

1. Mishra , A.,& Mohanty, B. (2008). Acute toxicity impacts of fluoride on the behavior and histopathology of fish Catla Catla. Acta Hydrochimica et Hydrobiologica, 36(5), 427-432.
   This study investigates the acute toxicity of fluoride on the behavior and tissue structure of the fish Catla catla, highlighting the histopathological changes.
2. Singh,M., Sandhir, R.,& Kiran, R.(2011). Fluoride induced oxidative stress in rats brain and its amelioration by buffalo (Bubalus bubalis) epiphyseal proteins. Biological Trace Element Research, 140(3), 354-362.
   While focusing on rats, this study provides insights into the oxidative stress mechanisms induced by fluoride, which are relevant to understanding similar processes in fish.

3. Seyfi, S., Ghaffari, H., Kheirkhah, M., & Sharifrohani, M. (2013). Antioxidant nd immunomodulatory effects of Aloe vera in rainbow trout (Oncorhynchus mykiss). Fish & Shellfish Immunology, 35(6), 2079-2083.
   This paper explores the antioxidant and immune- boosting properties of Aloe vera in rainbow trout, offering insights into its potential for mitigating oxidative stress caused by environmental toxins.
4. Nya, E.J.,& Austin,B.(2009). Use of dietary ginger, Zingiber officinale Roscoe, as an immunostimulant to control Aeromonas hydrophila infections in rainbow trout, Oncorhynchus mykiss (Walbaum).Journal of Fish Diseases, 32(11),971-977.
   While primarily focused on ginger, the study methodology and findings can be related to similar studies on Aloe vera, demonstrating how natural supplements can enhance fish health and recovery from stress.
5. Dadar,M., Thilakaratne, I.D.S.I.P., & Alzahrani, F.(2020). The potential application of Aloe vera in aquaculture: A review. Aquaculture International, 28(3), 977-990.
   This review discuss various application of Aloe vera in aquaculture, including its potential benefits for fish healh, diseases resistance, and recovery from environmental stressors.
6. Gupta, A.R., & Kushwah, S.(2016). Mitigating effects of dietary Aloe vera against fluoride- induced biochemical and hematological alterations in catfish (Heteropneustes fossilis). Ecotoxicology and Environmental Safety, 126,269-276.
   This Study directly addresses the mitigating effects of Aloe vera on fluoride- induced toxicity in catfish, providing experimental evidence of its protective properties.

7. Saha, S., & Kaviraj, A. (2009). Acute toxicity of fluoride to freshwater fish Channa punctatus and impact of calcium on fluoride toxicity. Indian Journal of Experimental Biology, 47(3),284-288.
   This research investigates the acute toxicity levels of fluoride in freshwater fish and examines how calcium influences toxicity, relevant for understanding mitigation strategies.

Significance of the study and its relevance in the context of current status:

Fish are crucial for both natural ecosystems and aquaculture industries. Fluoride toxicity can impair fish health, leading to economic losses in fisheries and aquaculture. Investigating natural remedies like Aloe vera can provide sustainable and cost- effective solutions to enhance fish resilience and promote recovery from environmental stressors.

The main concern is that Fish are an important part of the human diet, and fluoride accumulation in fish tissues can pose health risks to humans. Ensuring the health of fish through natural remedies not only improves fish welfare but also safeguards human consumers from potential fluoride exposure. There is a need to explore natural and effective methods to counteract the toxic effects of fluoride in fish. Aloe vera, known for its medicinal properties, presents a promising solution(Seyfi et al., 2013) also including its potential benefits for fish health, disease resistance, and recovery from environmental stressors (Dadar et al.,2020).

Research design and Methodology:

This study will use an experimental design with the following groups:

1. Control group (no fluoride exposure, no Aloe vera treatment)
2. Fluoride exposure group
3. Fluoride exposure + Aloe Vera treated group

Methodology:

1. To design a stimulated system of ambient water pH less than 5.6 and expose the test fishes in replicates of 20 individuals each for a period of 7 days at one fifth dose of LC50 dose of Sodium fluoride to induce physiological stress. Another group of fishes will be treated only with extract of Aloe vera for 7 days.
2. To treat the fishes under acute phase of toxicity with 1/5th dose of LC50 and also treat same lot fishes with Aloe vera extract to see the ameliorating agent treated individuals to assess to extent of damage as well as level of recovery.
3. Histopathological studies will be done for different tissues.
4. Biochemical assays- Blood samples will be collected to measure indicators such as oxidative stress markers, antioxidant enzymes and other relevant biochemical parameters.
5. To prepare SEM preparations of different tissues of control, fluoride treated, Aloe vera treated individuals to assess the extent of damage as well as level of recovery.

Year wise work plan:

1st Year

Literature survey
Design of experimental setup
Preparation of Aloe vera extract
Measurement of Biochemical parameters.

2nd Year

Histopathological study in different tissues
Measurement of antioxidant property of Aloe vera

3rd Year

SEM preparations of different tissues.
Statistical Analysis and data compilation

4th Year

Thesis writing and submission

5th Year

Final Viva – Voce

References:-

1. Camargo, J.A.(2003). Fluoride toxicity to aquatic organisms: A review. Chemosphere, 50(3),251-264.
2. Wang,A., et al.(2019). Molecular mechanisms of fluoride toxicity: A review on progress and prospects. Environmental Pollution,248,202-210.
3. Dey, D., & Giri,(2015). Toxicological effects of fluoride on fish. Aquatic Toxicology,165, 189-196.

4. Seyfi , S., Ghaffari, H., Kheirkhah, M., & Sharifrohani, M.(2013). Antioxidant and immunomodulatory effects of Aloe vera in rainbow trout(Oncorhynchus mykiss). Fish & Shellfish Immunology, 35(6), 2079-2083.
5. Dadar, M., Thilakaratne, I.D.S.I. P., & Alzahrani, F. (2020). The potential application of Aloe vera in aquaculture: A review Aquaculture International, 28(3), 977-990.
6. USEPA (2020). Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life. United States Environmental Protection Agency.
7. Ahmed, M.A., et al. (2019). Aloe vera as an Immunostimulant and Growth Promoter in Nile Tilapia (Oreochromis niloticus). Journal of Aquatic Animal Health.